

**COMPOSITIONS FOR THE DETECTION OF ENZYME ACTIVITY IN  
BIOLOGICAL SAMPLES AND METHODS OF USE THEREOF**

**ABSTRACT OF THE DISCLOSURE**

5           The present invention provides for novel reagents whose fluorescence  
increases in the presence of particular proteases. The reagents comprise a characteristically  
folded peptide backbone conjugated to two fluorophores such that the fluorophores are  
located opposite sides of a cleavage site.. When the folded peptide is cleaved, as by digestion  
with a protease, the fluorophores provide a high intensity fluorescent signal at a visible  
10       wavelength. Because of their high specificity and their high fluorescence signal in the  
visible wavelengths, these protease indicators are particularly well suited for detection of  
protease activity in biological samples, in particular in frozen tissue sections. Thus this  
invention also provides for methods of detecting protease activity *in situ* in frozen sections..